

21 Rec'd PCT/PT 29 DEC 1997

10584843024

08/983605

1

PCT/DE96/01185

2936.104/00

**MICROSATELLITE MARKERS FOR PLANTS OF THE SPECIES
TRITICUM AESTIVUM AND TRIBE TRITICEAE
AND THE USE OF SAID MARKERS**

BACKGROUND OF THE INVENTION

The invention relates to novel genetic markers for wheats (*Triticum aestivum* ~~L.~~) and closely related species (*Tribus: Triticeae*) ^{of the tribe} ^{of the species} and to the use of said markers.

The most widely spread, known, DNA-based genetic markers are the so-called restriction fragment length polymorphisms (RFLP) markers. For using these markers, genomic DNA is digested with restriction enzymes, separated on agarose gels and transferred to nylon membranes (Southern Blot). Specific fragments are detected by hybridization with radioactively labeled DNA probes. When mutations occur in the region of the restriction enzymes used or when smaller deletions/insertions occur, polymorphisms between different lines are found, which are passed on stably and mostly codominantly. The use of RFLP markers in hexaploid cultivated wheat is possible only to a limited extent, since only very little polymorphism is detected in wheat in this manner.

It has already been described that microsatellite markers detect significantly more polymorphism between different wheat lines than do RFLP markers. This can be attributed particularly to the occurrence of multiple alleles per locus (Röder et al., Mol. Gen. Genet. (1995) 246, 327 - 333). Moreover, it is known that microsatellite markers have the advantage that they can be detected by way of PCR and that therefore large amounts of samples can be analyzed more easily.

*Pfleiderer
Stalter*Summary of the Invention²

It is an object of the invention to provide novel microsatellite markers for the genetic analysis of plants of the Triticum aestivum species, which markers are distinguished by a degree of DNA polymorphism, which is higher than that of other molecular probes, that have been developed previously for the wheat genome.

9

DE 693 228 980

This objective is accomplished ~~by claims 1 to 10~~ *as follows*. The inventive markers are based on the amplification of certain hypervariable genome sections, the so-called microsatellites, with the help of their polymerase chain reaction (PCR). For specific amplification, two primers, in each case left and the right in the flanking sequences, are required for each microsatellite locus. On the average, these primers are 20 ± 3 bases long and are defined by their sequences. In principle, a microsatellite marker is a sequence tagged site (STS), which is defined by two specific primers. These primers flank, in each case to the left and the right, a so-called microsatellite sequence. A microsatellite sequence is defined as a tandem repetitive repetition of a di-, tri- or tetranucleotide sequence, for example $(GA)_n$, in which $n \geq 10$. Composite microsatellite sequences also occur, such as $(GT)_n(AT)_n$, as well as imperfect sequences, in which individual bases are mutated, such as $(GA)_nCA(GA)_n$. Among various lines and varieties, there is variation in the number of repeats at a certain locus. After amplification of the microsatellites, this leads, by means of the specific primers in the flanking sequences, to PCR products of different length and, with that, to polymorphisms. These polymorphisms are passed on stably and can therefore be used as genetic markers. In some cases, null alleles (no visible fragment) also occur, when there are mutations within the binding site for the primers.

The separation and detection of the PCR products obtained can be carried out with different technical variants. For separating the fragments, highly resolving agarose gels, native polyacrylamide gels or denaturing polyacrylamide gels (= sequencing gels) can be used. Depending on the separation system, fragments are detected using ethidium bromide staining, silver staining or, after labeling the PCR

fragments radioactively, using autoradiography. A further, very effective variation for separation and detection consists of the use of an automatic sequencer with dye- or fluorescence-labeled primers. For this purpose, it is necessary to synthesize a dye- or fluorescence-labeled primer from each microsatellite primer pair. PCR amplification results in a labeled product, which can be detected by the sequencing equipment. At the same time, dye- or fluorescence-labeled size standards are also separated for each sample in the same track. After that, special software enable the absolute size of each fragment, which has been separated, to be calculated and, with that, also permits fragments from different gel runs to be compared. With this method, several hundred samples can be analyzed largely automatically in a day.

DETAILED DESCRIPTION OF THE INVENTION

Pursuant to the invention, microsatellite markers are made available, which contain the following primer pairs with assigned microsatellite sequences or a number thereof and amplify the loci of all chromosomes of the wheat genome and therefore find use for gene marking.

BREWER - GENE MARKERS

1584843024

PCT/DE96/01185

WO 97/01567

Number Number	WMS WMS	Primer Left Primer Links	WMS WMS	Primer Right Primer rechts	Length (bp in "CS") Länge (bp) in 'CS"	Repeat-Typ Repeating- Type Temperatur Annealing- Temperatur Temperatur
52		5' CTA TGA GGG GGA GGT TGA AG 3'		5' TGC GGT GCT CTT CCA TTT 3'	150	GTimp
55		5' GCA TCT GGT ACA CTA GCT GCC 3'		5' TCA TGG ATG CAT CAC ATC CT 3'	127	CTimp
57		5' TGG ATT CTG AAA GGT TCA TCG 3'		5' CGA TCA AGT AGT TGA AAG CGC 3'	224	AAAAAimp
58		5' TCT GAT CCC GTG AGT GTA ACA 3'		5' GAA AAA AAT TGC ATA TGA GCC C 3'	118	CA
60		5' TGT CCT ACA CGG ACC ACG T 3'		5' GCA TTG ACA GAT GCA CAC G 3'	211	CA
63		5' TCG ACC TGA TCG CCG CTA 3'		5' CGG CCT GGG TGA TGA ATA GT 3'	271	GAA, CA, TA
67		5' ACC ACA CAA ACA AGG TAA GCG 3'		5' CAA CCC TCT TAA TTT TGT TGG G 3'	85	CA
68		5' AGG CCA GAA TCT GGG AAT G 3'		5' CTC CCT AGA TGG GAG AAG GG 3'	182	GA
70		5' AGT GGG TGG GAG AGT GTC AT 3'		5' GCC CAT TAC CGA GGA CAC 3'	194	GT
71		5' GGC AGA GCA GCG AGA CTC 3'		5' CAA GTG GAG CAT TAG GTA CAC G 3'	128	GT
77		5' ACA AAG GTA AGC AGC ACC TGT 3'		5' ACC CTC TPG CCC GTG TTG 3'	153	CA, GA
82		5' ACG TTA GAA GGT GCA ATG GG 3'		5' AGT GGA TGC AGC GAC TTT G 3'	152	GT, GAimp
88		5' CACT TAC AAC TAT GCG CTC GC 3'		5' TCC ATT GGC TTC TCT STC AA 3'	121	GT
95		5' GAT CAA ACA CAC ACC CCT CC 3'		5' AAT GCA AAG TGA AAA ACC SG 3'	121	CA
99		5' AAG ATG GAC GTA TGC ATC ACA 3'		5' GCC ATA TTT GAT GAC GCA TA 3'	119	CA
102		5' TCT CCC ATC CAA CGC CTC 3'		5' TGT TGG TGG CTT GAC TAT TG 3'	143	CT
106		5' CTG TTC TTG CGT GGC ATT AA 3'		5' AT AAG GAC ACA ATT GGG ATG G 3'	139	GA
107		5' ATT AAT ACC TGA GGG AGG TGC 3'		5' AAT AGG AGC AAG AAC AC 3'	195	CT
108		5' CGA CAA TGG GGT CTT AGC AT 3'		5' GGT CTC AGG AGC AAG AAC AC 3'	132	CTimp
111		5' TCT GTA GGC TCT CTC CGA CTG 3'		5' TGC ACA CTT AAA TTA CAT CCG C 3'	205	CT, GT
112		5' CTA AAC ACG ACA CGG GTG G 3'		5' ACC TGA TCA GAT CCC ACT CG 3'	101	CTimp
113		5' ATT CGA GGT TAG GAG GAA GAG G 3'		5' GAT ATG TGA GCA GCG GTC AG 3'	148	GT
114		5' ACA AAC AGA AAA TCA AAA CCC G 3'		5' ATC CAT CGC CAT TGG AGT G 3'	206	GA
					(177)	
3118		5' GAT GTT GCC ACT TGA GCA TG 3'		5' GAT TAG TCA AAT GGA ACA CCC C 3'	110	CA
3119		5' TGA CTA ACA TCC TTT GTG ACG C 3'		5' CAT GTC TCA ACC ACC CAC AG 3'	181	GTimp

8484302

PCT/DE96/01185

WO 97/01567

MS120	5' GAT CCA CCT TCC TCT CTG TC 3'	5' GATTAT ACT GGT GCC GAA AC 3'	139	CT, CA	55 °C
MS121	5' TCC TCT ACA AAC AAA CAC AC 3'	5' CTC GCA ACT AGA GGT GTA TG 3'	143	CA	50 °C
MS122	5' GGG TGG GAG AAA GGA GAT G 3'	5' AAA CCA TCC TCC ATC CTG G 3'	149	CT, CA	60 °C
MS124	5' GCC ATG GCT ATC ACC CAG 3'	5' ACT GTT CGG TGC AAT TTG AG 3'	213	CT, GTimp	60 °C
MS126	5' CAC ACG CTC CAC CAT GAC 3'	5' GTT GAG TTG ATG CGG GAG G 3'	196	CA	60 °C
MS128	5' AGC ACA TTT TAA CAC AGA TA 3'	5' ATC TGT GAA ATT TTG AAA AC 3'	176	CA	50 °C
MS129	5' TCA GTG GGC AAG CTA CAC AG 3'	5' AAA ACT TAG TAG CCG CGT 3'	221	GTimp	55 °C
MS130	5' AGC TCT GCT TCA CGA GGA AG 3'	5' CTC CTC TTT ATA TCG CGT CCC 3'	113	GT	60 °C
MS131	5' AAT CCC CAC CGA TTC TTC TC 3'	5' AGT TGT TGG GTC TCT GAT GG 3'	131	CT	60 °C
MS132	5' TAC CAA ATC GAA ACA CAT CAG G 3'	5' CAT ATC AAG GTC TCC TTC CCC 3'	119	GA, GAA	60 °C
MS133	5' ATC TAA ACA AGA CGG CGG TG 3'	5' ATC TGT GAC AAC CGG TGA GA 3'	118	CT	60 °C
MS134	5' CAT GGA ACT TAG ACA GAA TTG 3'	5' CAG TAC TTG GTA CTG AAC AGG 3'	111	CA	60 °C
MS135	5' TGT CAA CAT CGT TTT GAA AAG G 3'	5' ACA CTC TCA ACC TGG CAA TG 3'	143	GA	55 °C
MS136	5' GAC AGC ACC TTG CCC TTT G 3'	5' CAT CGG CAA CAT GCT CAT C 3'	296	CT	60 °C
MS140	5' ATG GAG ATA TTT GGC CTA CAA C 3'	5' CTT GAC TTC AAG GCG TGA CA 3'	251	CT	55 °C
MS144	5' TTT GCT GTG GTA CGA AAC ATA C 3'	5' ACT CAC AAA TGT CTA ATA AAA C 3'	200	GT	50 °C
MS146	5' CCA AAA AAA CTG CCT GCA TG 3'	5' CTC TGG CAT TGC TCC TTG G 3'	162	GAimp	60 °C
MS148	5' GTG AGG CAG CAA GAG AGA AA 3'	5' CAA AGC TTG ACT CAG ACC AAA 3'	163	CA	60 °C
MS149	5' CAT TGT TTT CTG CCT CTA GCC 3'	5' ACT CAC AAA TGT CTA ATA AAA C 3'	161	GA	55 °C
MS153	5' GAT CTC GTC ACC CGG AAT TC 3'	5' CAA TGG TAG AGA AGG AGC GAG AG 3'	188	GA	60 °C
MS154	5' TCA CAG AGA GAG AGG GAG GG 3'	5' TGG TAG AGA AGG AGC GAG AG 3'	102	GA	55 °C
MS155	5' CAA TCA TTT CCC CCT CCC 3'	5' ATG TGT ACA TGT TGC CTG CA 3'	141	CT	60 °C
MS156	5' CCA ACC GTG CTA TTA GTC ATT C 3'	5' GCA TGG AGG CCC TCC TAA C 3'	277	GT	60 °C
MS157	5' GTC GTC GCG GTA AGC TTG 3'	5' GAG TGA ACA CAC GAG GCT TG 3'	106	CT	60 °C
MS159	5' GGG CCA ACA CTG GAA CAC 3'	5' GCA GAA GCT TGT TGG TAG GC 3'	192	GT	60 °C
MS160	5' TTC AAT TCA GTG TTG GCT TTG 3'	5' CTG CAG GAA AAA AAG TAC ACC C 3'	184	GA	60 °C
MS161	5' GAT CGA GTG ATG GCA GAT GG 3'	5' TGT GAA TTA CTT GGACGT GG 3'	154	CT	60 °C
MS162	5' AGT GGA TCG ACA AGG CTC TG 3'	5' AGA AGA AAA GCC TTC CC 3'	208	CA	60 °C

M584843024

PCT/DE96/01185

WO 97/01567

MSI163	5' ACC TCG ACA GAC CTC GTA CG 3'	5' GTC TTT GTC ACC CGA TGG AC 3'	127	CT	55 °C
MSI164	5' ACA TTT CTC CCC CAT CGT C 3'	5' TTG TAA ACA AAT CGC ATG CG 3'	120	CT	55 °C
MSI165	5' TGC AGT GGT CAG ATG TTT CC 3'	5' CTT TTC TGT CAG ATT GCG CC 3'	199	GA	60 °C
MSI166	5' ACC ACT GCA GAG AAC ACA TAC G 3'	5' GTG CTC TGC TCT AAG TGT GGG 3'	196	GA	60 °C
MSI174	5' GGG TTC CTA TCT GGT AAA TCC C 3'	5' GAC ACA CAT GTT CCT GCC AC 3'	173	CT	55 °C
MSI179	5' AAG TTG AGT TGA TGC GGG AG 3'	5' CCA TGA CCA GCA TCC ACT C 3'	181	GT	55 °C
MS180	5' ATC CGC CTA AGG AAT ATG GT 3'	5' GAT CGC ACG GGA GAG AGA G 3'	84	CT	50 °C
MS181	5' TCA TTG GTA ATG AGG AGA GA 3'	5' GAA CCA TTC ATG TGC ATG TC 3'	135	GA	50 °C
MS182	5' TGA TGT AGT GAG CCC ATA GGC 3'	5' CGC CTC TAG CGA GAG CTA TG 5'	165	CT	60 °C
MS186	5' GCA GAG CCT GGT TCA AAA AG 3'	5' GAA AT ACG GAA ACC CAC CC 3'	140	GA	55 °C
MS189	5' AGG AGC AGG GGA ACG AAC 3'	5' AGA AAT GCA CGT GGT ACC TTT G 3'	117	CA	60 °C
MS190	5' GTG CTR GCT GAG CTA TGA GTC 3'	5' TAG CAC GAC AGT TGT ATG CAT G 3'	>201	CT,GT	60 °C
MS191	5' AGA CTG TTG TTT GCG GGC 3'	5' CGT TGT CTA ATCT TIG CCT TGC 3'	128	CT	60 °C
MS192	5' GGT TTT CTT TCA GAT TGC GC 3'	5' AAT TGT GTT GAT GAT TTG GGG 3'	191	CT	60 °C
MS193	5' CTT TGT GCA CCT CTC TCT CC 3'	5' CGA CGC AGA ACT TAA AGA AG 3'	171	CT,CA	60 °C
MS194	5' GAT CTG CTC TAC TCT CCT CC 3'	5' CGA CCG CAC GTC AGA GAG 3'	131	CT	50 °C
MS195	5' AGG TGC CGT CGC GTCTAC 3'	5' ACC CCC CAC GTC AGA GAG 3'	108	CT	60 °C
MS197	5' GAG AAA GAG GTC TGG AGG TCG 3'	5' CAA AAT GCA CAA GAA TGG AGG 3'	126	CT	60 °C
WMS198	5' TTG AAC CGG AAG GAG TAC AG 3'	5' TCA GTT TAT TTG GGT CAT GTG 3'	130	CA	60 °C
WMS200	5' TCA ACG GAA CAG ATG AGC G 3'	5' GAC CTG ATG AGA GCA AGC AC 3'	250	CT	55 °C
WMS203	5' CCC AAA GCA GCG CAA GC 3'	5' ACC AAT GCT ATC GGC TCG 3'	139	CA,GA	60 °C
WMS205	5' CGA CCC GGT TCA CTT CAG 3'	5' AGT CGC CGT TGT ATA GTG CC 3'	152	CT	60 °C
WMS210	5' TGC ATC AAG AAT ATG GTG GAA G 3'	5' TGA GAG GAA GGC TCA CAC CT 3'	192	GA	60 °C
WMS212	5' AAG CAA CAT TTG CTG CAA TG 3'	5' TGC AGT TAA CTT GTT GAA AGG A 3'	104	CT	60 °C
WMS213	5' TGC CTG GCT CGT TCT ATC TC 3'	5' CTA GCT TAC CAC TGT CGC CC 3'	184	GA	60 °C
WMS218	5' CGG CAA ACG GAT ATC GAC 3'	5' AAC AGT AAC TCT CGC CAT AGC C 3'	149	CT	60 °C
WMS219	5' GAT GAG CGA CAC CTA GCC TC 3'	5' GGG GTC CGA GTC CAC AAC 3'	181	GAimp	60 °C
WMS224	5' TGA GTC CAG CAC TGC TGC 3'	5' CAA CAT CCC CTC GTA TTC AA 3'	142	CT	50 °C

M58484302

PCT/DE96/01185

WO 97/01567

MS228	5' TCA TAT GCA CCT CTT TCC TAGG 3'	5' GTG TGC CAC CCT TGA CCT C 3'	210	CT,CA	60 °C
MS231	5' AGC TCG GGA TGA AGC GTG 3'	5' GAT CCG CCG CTG CGT TT 3'	130	GAimp	60 °C
MS232	5' ATC TCA ACG GCA AGC CG 3'	5' CTG ATG CAA GCA ATC CAC C 3'	141	GA	55 °C
MS233	5' TCA AAA CAT AAA TGT TCA TTG GA 3'	5' TCA ACC GTG TGT AAT TTT GTG C 3'	261	CT	60 °C
MS234	5' GAG TCC TGA TGT GAA GCT GTT G 3'	5' CTC ATT GGG GTG TGT ACG TG 3'	241	CT,CA	55 °C
MS237	5' GAA TCA CTT GTG AAG CAT CTG G 3'	5' CTG GAT GCA TCA CAT CCA AC 3'	137	CT	55 °C
MS238	5' TCG CTT CTA CCG CTC ACC 3'	5' AGT GCC TTG CCG AGG TC 3'	204	CT,GT,GGGT	55 °C
MS241	5' TCT TCC AAC TAA AGC ATA GC 3'	5' CTT CCA TGG ACT ACA TAC TAG C 3'	146	GA	55 °C
MS242	5' TCC AAG GCA GTA GGC AGG 3'	5' TGT TGT CCT GTA TGC AT 3'	142	GA	55 °C
MS244	5' GGC AGC TGA GGC AAT CTG 3'	5' TGT GGA CAT TTC CCA GCG 3'	227	CAimp	60 °C
MS245	5' CAG CGC AGT TAG CTC GC 3'	5' TTT GGA ATG TCG GAC GC 3'	141	CT	60 °C
MS247	5' GCA ATC TTT TTT CTG ACC ACG 3'	5' ATC TGT CCA TTC GAG CGC 3'	158	GA	60 °C
MS248	5' AGG ACT TCC GCA CCC TG 3'	5' ATG TGC ATG TCG GAC GC 3'	185	CA	60 °C
MS249	5' CAA ATG GAT CGA GAA AGG GA 3'	5' TGG CGT GGT CTA AAT GGA C 3'	177	GAimp	60 °C
MS251	5' CAA CTG GTT GCT ACA CAA GCA 3'	5' CTG CCA ATT TTC TGG ATC TAC C 3'	103	CA	55 °C
MS255	5' CAA CTG TAC GTA GGT TTC ATT GC 3'	5' GGG ATG TCT GTT CCA TCT TAG 3'	148	GA	55 °C
MS257	5' AGA GTG CAT GGT GGG ACG 3'	5' TCT GCC GTA AGT CGC CTC 3'	192	GT	60 °C
MS258	5' GAT CGC TTC ATC TCT TCT C 3'	5' CCA AGA CGA TGC TGA AGT CA 3'	>81	CT	60 °C
MS259	5' AGG GAA AAG ACA TCT TTT TTT TC 3'	5' GTA CAC GCC GTA GGC CC 3'	105	GA	55 °C
MS260	5' GCC CCC TTG CAC AAA TCC 3'	5' CGA CCG ACT TCG GGT TCC 3'	157	GA	55 °C
MS261	5' CTC CCT GTA CGG CTA AGG C 3'	5' CGC AGC TAC AGG AGG CC 3'	192	CT	55 °C
MS263	5' TCT GCC GTA AGT CGC CTC 3'	5' CTC GCG CTA CTA GCC ATT G 3'	134	CT	55 °C
MS264	5' GAG AAA CAT GCC GAA CAA CA 3'	5' GGT TTC ATT GCT TGC CCT AA 3'	219	CA	60 °C
MS265	5' TGT TGC GGA TGG TCA CTA TT 3'	5' GCA TGC ATG AGA ATA GGA ACT G 3'	200	GT	55 °C
MS268	5' AGG GGA TAT GTT GTC ACT CCA 3'	5' GAG TAC ACA TTT GGC CTC TGC 3'	241	GAimp	60 °C
MS269	5' TGC ATA TAA ACA GTC ACA CAC CC 3'	5' TTA TGT GAT TGC GTA CGT ACC C 3'	>148	CA	55 °C
MS271	5' CAA GAT CGT GGA GCC AGC 3'	5' TTT GAG CTC CAA AGT GAG TTA GC 3'	162	CT,GA	60 °C
MS272	5' TGC TCT TTG GCG AAT ATA TGG 3'	5' GTC CAA AAC AAA TTA AAA GGC CC 3'	140	CA	55 °C

M58484302

WO 97/01567

PCT/DE96/01185

			8
S273	5' ATT GGA CGG ACA GAT GCT TT 3'	5' AGC AGT GAG GAA GGG GAT C 3'	167 GA
S274	5' AAC TTG CAA AAC TGT TCT GA 3'	5' TAT TTG AAG CGG TTT GAT TT 3'	179 GT
S275	5' AAT TTT CCT CCT CAC TTA TTCT 3'	5' AAC AAA AAA TTA GGG CC 3'	107 CT
S276	5' ATT TGC CTG AAG AAA ATA TT 3'	5' AAT TTC ACT GCA TAC ACA AG 3'	99 CT
S277	5' GTC GCT TCA TGA ACG CTC AA 3'	5' CTG CCC AAT TTT CTC CAC TC 3'	241 GTimp/GAimp
S278	5' CGG CCA TAT TTC TGT AAG TAT GC 3'	5' GCA GGT AAT GGC CGG AC 3'	135 GT
S281	5' TTG GCC GTG TAA GGC AG 3'	5' TCT CAT TCA CAC ACA ACA CTA GC 3'	220 GA
S282	5' AAT GAA AAA ACA CTT GCG TGG 3'	5' GCA CAT TTT TCA CTT TCG GG 3'	123 GA
S284	5' ATG ACC CTT CTG CCA AAC AC 3'	5' ATC GAC CGG GAT CTA GCC 3'	243 GA
S285	5' CAT CCC TAC GCC ACT CTG C 3'	5' AAT GGT ATC TAT TCC GAC CCG 3'	>158 CA
S291	5' TCA CCG TGG TCA CCG AC 3'	5' CCA CCG AGC CGA TAA TGT AC 3'	220 CT
S292	5' TAC TGG TTC ACA TTG GTG CG 3'	5' TCG CCA TCA CTC GTT CAA G 3'	201 CA
S293	5' GGA TTG GAG TTA AGA GAG AAC CG 3'	5' GCA GAG TGA TCA ATG CCA GA 3'	100 GAimp
S294	5' GTG AAG CAG ACC CAC AAC AC 3'	5' GAC GGC TGC GAC GTA GAG 3'	258 GA
S295	5' AAT TCA ACC TAC CAA TCT CTG 3'	5' GCC TAA TAA ACT GAA AAC GAG 3'	149 CT
S296	5' ATC GTC ACG TAT TTT GCA ATG 3'	5' TGC GTA AGT CTA GCA TTT TCT G 3'	150 GT, GA
S297	5' ACT ACT TAG GCC TCC CGC C 3'	5' TGA CCC ACT TGC AAT TCA TCC 3'	208 GA, TAG
S299	5' GAG GAG TAA GAC ACA TGC CC 3'	5' GTG GCT GGA GAY TCA GGT TC 3'	204 GA, G
S301	5' GCA AGA AGC AAC AGC AGT AAC 3'	5' CAG ATG CTC TTC TCT GCT GG 3'	180 (340) GA
S302	5' AGG AAA CAG AAA TAT CGC GG 3'	5' AGG ACT GTG GGG AAT GAA TG 3'	217 CT
S304	5' CGG CCC TCA TTA AGT TTC AC 3'	5' CTA CGT GCA CCA CCA TTT TG 3'	151 GA
S311	5' AGG AGC TCC TCT GTG CCA C 3'	5' ACA TGC ATG CCT ACC TAA TGG 3'	235 GA
S312	5' ATC GCA TGA TGC ACG TAG AG 3'	5' TGT GTC TGG TCC ACC TC 3'	176 CT,GT
S313	5' CAT GGA CAT TTT ACC ACA AGA C 3'	5' TTT GAC AAG TAC ACG AGT CTG C 3'	170 CT
S314	5' CGG GTG CTG TGT GTA ATG AC 3'	5' TTC GGG ACT CTC TTC CCT G 3'	176 AT,GT
S316	5' GGT TGC TGT ACA AGT GTT CAC G 3'	5' CGG GTG CTG TGT GTA ATG AC 3'	200 CT
S319	5' CGA GAT ACT ATG GAA GGT GAG G 3'	5' ATC TTT GCA AGG ATT GCC C 3'	>263 GT, GA
S320	5' CAA TGT GGA GAC GGT GTG C 3'	5' TGT TGC ATG CGA TCA TGC 3'	265 GT, GAimp

M 58 484302

PCT/DE96/01185

WO 97/01567

9

S322	5' TCA CAA AAT GAT TTC TCA TCC G 3'	5' TGC AGA AAA CCA ACA AGG G 3'	119	GA	55 °C
S325	5' TTT CTT CTG TCG TTC TCT TCC C 3'	5' TTT TTA CGC AAC GAC G 3'	131	CT	55 °C
S328	5' GCA ATC CAC GAG AAG AGA GG 3'	5' CAC AAA CTC TTG ACA TGT GCG 3'	193	GT	55 °C
S330	5' TTG CTA TCC ATG TGC CAG AG 3'	5' ACA TGT TTC ATG CAG GTA GCC 3'	165	GTT	55 °C
S332	5' ACC CAG CAA GTC ACC AAA AC 3'	5' AGT GCT GGA AAG AGT AGT GAA GC 3'	231	GA	60 °C
S333	5' GCC SGG TCA TGT AAA ACG 3'	5' TTT CAG TTT GCC TTA AGC' TTT G 3'	150	GA	55 °C
S334	5' AAT TTC AAA AAG GAG AGA GA 3'	5' AAC ATG TGT TTT TAG CTA TC 3'	123	GA	50 °C
S335	5' CGT ACT CCA CTC SAC ACG G 3'	5' CGG TCC AAG TGCTAC CTT TC 3'	187 (225)	GA, GCGT	55 °C
S336	5' CCC TTT AAT CTC GCT CCTS TC 3'	5' GTCTCTTTCG TAC TTC CAG G 3'	108	CT	55 °C
S337	5' CCT CTT CCT CCC TCA CTT AGC 3'	5' TGCTAACTG GCT TTT GCC 3'	183	CT, CACT, CA	50 °C
S339	5' AAT TTT CCT CCT CAC TTA TT 3'	5' CGG AAA CGA ACA ACC ACT CAA TC 3'	159	CT	55 °C
MS340	5' GCA ATC TTT TTT CTG ACC ACG 3'	5' ACC AGG CAA GAA CAC ACA TG 3'	132	GA	60 °C
MS341	5' TTC AGT GGT AGC GGT CGA G 3'	5' ACC AGG CAA GAA CAC ACA TG 3'	133 (150)	CT	55 °C
MS342	5' TAT CCA GAG CAG ACG GAC G 3'	5' CGG AAC ATCT CAT GGA TCC AC 3'	169	GT	55 °C
MS344	5' CAA GGA AAT AGG CGG TAA CT 3'	5' GCA TGT GGT CCA TGT ACT AC 3'	131	GT	55 °C
MS346	5' CAA GCA AGG TTT CGT TTT ATC C 3'	5' ATT TGA GTC TGA AGT TTG CA 3'	203	AT, GT	55 °C
MS349	5' GGC TTC CAG AAA ACA ACA GG 3'	5' GCA TGG ATA CGA CGC CC 3'	230	GA	55 °C
MS350	5' ACC TCA TCC ACA TGT TCT ACG 3'	5' GCA TGG ATA CGA CGC CC 3'	146	GT	55 °C
MS353	5' CCA TGT TGA GTA GGT TCA GCC 3'	5' CTT GCC CAG AAG CTA CGA AC 3'	179	GGGT, GT	60 °C
MS356	5' AGC GTT CCT GGG AAT TAG AGA 3'	5' CCA ATC AGC CTG CAA CAA C 3'	224	GA	55 °C
MS357	5' TAT GGT CAA AGT TGG ACC TCG 3'	5' TCC GCT GTT GTT CTG ATC TC 3'	123	GA	55 °C
MS358	5' AAA CAG CGG ATT TCA TCG AG 3'	5' TAC TGT TGT TCT GGG ACA ATG G 3'	164	GAimp	55 °C
MS359	5' CTA ATT GCA ACA GGT CAT GGG 3'	5' AGG CTG CAG CTC TTC TTC AG 3'	217	CT, CTTimp	55 °C
MS361	5' GTA ACT TGT TGC CAA AGG GG 3'	5' TCC GCT GTT GTT CTG ATC TC 3'	126	GAimp	60 °C
MS368	5' CCA TTT CAC CTA ATG CCT GC 3'	5' AAT AAA ACC ATG AGC TCA CTT GC 3'	144	AT	60 °C
MS369	5' CTG CAG GCC ATG ATG ATG 3'	5' ACC GTG GGT GTT GTG AGC 3'	188	CTimp	60 °C
MS371	5' GAC CAA GAT ATT CAA ACT GGC C 3'	5' AGCTCA GCT TGC TTT GTA CC 3'	170	CA, GA	60 °C
MS372	5' AAT AGA GCC CTG GGA CTG GG 3'	5' GAA GGA CGA CAT TCC ACC TG 3'	>329	GA	60 °C

M 5 8 4 8 4 3 0 2

PCT/DE96/01185

WO 97/01567

10

4	5' ATA GTG TGT TGC ATG CTG TGT G 3'	5' TCT AAT TAG CGT TGG CTG CC 3'	213	GT	60 °C
5	5' ATT GGC GAC TCT AGC ATA TAC G 3'	5' GGG ATG TCT GTT CCA TCT TAG C 3'	156	CA	55 °C
15	5' GGG CTA GAA AAC AGG AAG GC 3'	5' TCT CCC GGA CCA CCA TTT TG 3'	147	CA, GAimp	60 °C
16	5' GTC AGA TAA CGC CGT CCA AT 3'	5' CTA CGT GCA CCA CCA TTT TG 3'	115	GA	60 °C
32	5' ACC CCA GTT GAT CCG TAA AC 3'	5' GAC ATC AT AT AAC CGT GGA TGG 3'	195	GT	60 °C
33	5' TTT TCA TTG TGC CCT CTA CT 3'	5' GCC AAG TTT CTT AGC TAG TTA A 3'	204	GTimp	55 °C
84	5' CTA CAA TAC GAA GGA GAG GGG 3'	5' CAC CGC GTC AAC TAC TTA AGC 3'	162	CT, CA, CA	60 °C
88	5' ATC ATG TCG ATG TCC TTG ACG 3'	5' TGC CAT GCA CAT TAG CAG AT 3'	130	CT, GT	60 °C
89	5' AAG TTT CAC ACA AGA TCT CTC C 3'	5' TGA CAA GTA CAC GAG TCT GC 3'	143	CT, GT	55 °C
90	5' ATA GCG AAG TCT CCC TAC TCC A 3'	5' ATG TGC ATG TCG GAC GC 3'	150	CA, GA	55 °C
91	5' TCA TCT GCT ATT TGT GCT ACA 3'	5' TCA AAT ACA CCA ATG TGC C 3'	107	CA	55 °C
93	5' TAC AAC CGC AAG TAA TGC CA 3'	5' TAC CAA CAC CCT AGC CCT TG 3'	147	CA	60 °C
95	5' TGT CAT GGA TTA TTT GGT CGG 3'	5' CTG GAC TCT CGG TAT ACC AGC 3'	179	CT	55 °C
97	5' GTG CTG CCA CCA CTT GC 3'	5' TGT AGG TAC TGC TTG GGA G 3'	139	CA	60 °C
100	5' CGA CAT TGG CTT CGG TG 3'	5' ATA AAA CAG TGC GGT CCA GG 3'	133	CA	55 °C
103	5' TCG ATT TAT TTG GGC CAC TG 3'	5' GAA TAA TTC GTT CAC AGC ACG C 3'	176	CA	55 °C
408	5' GCT TGA GAC CGG CAC AGT 3'	5' GAA TAA TTC GGT CCT GGT CCC 3'	334	CA	55 °C
410	5' CCC ATA CGA TGA TGT GTT TCC 3'	5' CAA ACG GAA CAT GGT CCC 3'	148	CT	55 °C
411	5' ATC AAC AAG GTT TGT GTT CTT GGG 3'	5' ATG AAA CGG CAC CCC CC 3'	121	CA	55 °C
412	5' TGC TTG TCT AGA TTG CTT GGG 3'	5' GAT CGT CTC GTC CCT GGG A 3'	94	GA	60 °C
413	5' GAT CTC CCA TGT CCG CC 3'	5' CGA CAG TCG TCA CCT GCC TA 3'	131	GAimp	55 °C
415	5' CGA GGC AGC GAG GAT TT 3'	5' TCG TTC TCC CAA GGC TTG 3'	>143	CT	60 °C
425	5' GAG CCC ACA AGC TGG CA 3'	5' AGT GTG TTC ATT TGA CAG TT 3'	215	CA	50 °C
427	5' AAA CTT AGA ACT GTA ATT TCA GA 3'	5' TTC TCC ACT AGC CCC GC 3'	143	GA	55 °C
428	5' TTG TAC ATT AAG TTC CCA TTA 3'	5' TTT AAG GAC CTA CAT GAC AC 3'	221 (290)	CT	50 °C
5429	5' ATG AGT TCC GCC AAA GAA TG 3'	5' AGC AAA TAC ACA AGT GGG ACA 3'	216	GT	55 °C
5434	5' GAT CAA GAC CAG TTA GCT TA 3'	5' GAT GTC CAA CAG TTA GCT TA 3'	109	CT	50 °C
5437	5' CCT ATG GTC TCC ATC ATG AGG 3'	5' TCA TGT CAA CTC AAC AAC ACG 3'	112	CT	55 °C
5440					

58 484302

PCT/DE96/01185

WO 97/01567

11

MS443	5' GGG TCT TCA TCC GGA ACT CT 3'	5' CCA TGA TTT ATA AAT TCC ACC 3'	134	CA, GA	55 °C
MS445	5' TTT GGT GGG GGT TAG GAT TAG 3'	5' CCT TAA CAC TTG CTG GTA GTG A 3'	192	CT	55 °C
MS448	5' AAA CCA TAT TGG GAG GAA AGG 3'	5' CAC ATG GCA TCA CAT TTG TG 3'	231	GA	60 °C
MS448	5' ATT CGG TTC GCT AGC TAC CA 3'	5' ACG GAG AGC AAC CTG CC 3'	151	GTemp	55 °C
MS455	5' TCT GAA CAT TAC ACA ACC CTG A 3'	5' TGC TCT TGA ACC TGA AGC 3'	132	GA	55 °C
MS456	5' AAT GGS AAT TGG AAG ACA TAG C 3'	5' TTC GCA ATG TTG ATT TTG C 3'	113	CA	60 °C
MS458	5' ATG GAG TGG TCA CAC TTT GAA 3'	5' AGC TTCTCT GAC CAA CTT CRC GC 3'	>138	GA	55 °C
MS459	5' CAA CTC AGT GCT GAC ACA ACG 3'	5' CGA TAA CCA CTC ATC CAC ACC 3'	>156	CT	60 °C
MS469	5' CGG CCC TAT CAT GGCT GS 3'	5' GCT TGC AAG TTC CAT TTT GC 3'	149	CA	60 °C
MS471	5' TCA TAC GGG TAT GGT TTG AC 3'	5' CAC CCC CTT GTT GGT CAC 3'	220	GTemp	55 °C
MS473	5' ATG GGT TCG TAC TAA CAT CAGC 3'	5' TTG CTG GTC GCT TCA ATC CC 3'	>194	GAimp	60 °C
MS476	5' TGC TGC TAC TTG TAC AGA GGA C 3'	5' CCG AAT TGT CCG CCA TAG 3'	188	CT, CA	60 °C
MS480	5' ACA TCG CTC TTC ACA AAC CC 3'	5' AGT TCC GGT CAT GGC TAG G 3'	145	CT	55 °C
MS484	5' ATT GAA CAG GAA GAC ATC AGG G 3'	5' TIC CTG GAG CTG TCT GGC 3'	198	CA	60 °C
MS494	5' GAG AGC CTC GCG AAA TAT AGG 3'	5' TGC TTC TGG TGT TCC TTC GC 3'	168	GA	60 °C
MS495	5' GTA GTG AAG ACA AGG GCA TT 3'	5' CCG AAA GTT GGC TGAT AT AC 3'	>106	GTemp	55 °C
MS497	5' ACT TGT ATG CTC CAT TGA TTG G 3'	5' GGG GAG TGG AAA CTG CAT AA 3'	145	GA	60 °C
MS499	5' GGCT TAT CTC TGG CGC TAA AA 3'	5' TCC ACA AAC TAG TAG CGC C 3'	172	CA	60 °C
MS501	5' AGC CAC CAT CAG CAA AAA TT 3'	5' GAA CAT GAG CAG TTT GGC AC 3'	185	GT	60 °C
MS512	5' AAT CAC AAC AAG GCG TGA CA 3'	5' GGT CTG TTC ATG CCA CAT TG 3'	144	CA	60 °C
MS513	5' ATC CGT AGC ACC TAC TGG TCA 3'	5' CCT TCA ACT TCT TGG CCT CCA TC 3'	134	GTemp	60 °C
MS515	5' AAC ACA ATG GCA AAT GCA GA 3'	5' CAG GGT GGT GCA TGC AT 3'	166	CA	55 °C
MS518	5' AAA TAG GAC AAC CCA CGG C 3'	5' TCA ACT TCT TGG CCT CCA TC 3'	186	CT	55 °C
MS530	5' ACT GCG TGT GCC TAC AAT TG 3'	5' TCA CTC GCA CTC GAT AGG C 3'	142	GT	60 °C
MS532	5' AAG GCG AAT CAA ACG GAA TA 3'	5' GTT GCT TTA GGG GAA AAG CC 3'	147	CT, CA	60 °C
VMS533	5' ACA TAA TGC TTC CTG TGC ACC 3'	5' GCC ACT TTT GTG TCG TTC CT 3'	209	CA, TA	60 °C
VMS537	5' GCA TGT ATA CGT TAA GCG G 3'	5' GTT GCA TGT ATA CGT TAA GCG G 3'	147	GTemp	60 °C
VMS538	5' TCT CGC TGT GAA ATC CTA TTT C 3'	5' AGG CAT GGA TAG AGG GGC 3'	129	GTemp	55 °C
VMS540					

WO 97/01567

WMS544	5' TAG AAT TCT TTA TGG GGT CTG C 3'	5' AGG ATT CCA ATC CTT CAA AATT 3'	167	CT, ATCT, CT	55 °C
WMS550	5' CCC ACA AGA ACC TTT GAA GA 3'	5' CAT TGT GTG TGC AAG GCA C 3'	150	CT, GT	55 °C
WMS554	5' TGC CCA CAA CGG AAC TTG 3'	5' GCA ACC ACC AAG CAC AAA GT 3'	160	CT, GTimp	60 °C
WMS555	5' GCG TCA GAT ATG CCT ACC TAG G 3'	5' AGT GAG TTA GCC CTG AGC CA 3'	142	CA	60 °C
WMS566	5' TCT GTCTAGCCA TGG GAT TTG 3'	5' CTG GCT TCG AGG TAA GCA AC 3'	130	CA, TA	60 °C
WMS569	5' GGA AAC TTA TTG ATT GAA AT 3'	5' TCA ATT TTG ACA GAA GAA TT 3'	134	GT	47 °C
WMS570	5' TCG CCT TTT ACA GTC GSC 3'	5' ATG GGT AGC TGA GAG CCA AA 3'	143	CT, GT	60 °C
WMS573	5' AAG AGA TAA CAT GCA AGA AA 3'	5' TTC AAA TAT GTG GGA ACT AC 3'	212	CA	50 °C
WMS577	5' ATG GCA TAA TTT GGT GAA ATT G 3'	5' TGT TTC AAG CCC AAC TTC TAT T 3'	133	CA, TA	55 °C
WMS582	5' AAG CAC TAC GAA AAT ATG AC 3'	5' TCT TAA GGG GTG TTA TCA TA 3'	151	CA	50 °C
WMS583	5' TTC ACA CCC AAC CAA TAG CA 3'	5' TCT AAG CAG ACA CAT GCC TG 3'	165	CA	60 °C
WMS588	5' GAT CCC CAA TTG CAT GTT G 3'	5' CTT GCA ACT GGG GCA CAC 3'	102	GT	60 °C

'CS' Weizensoric 'Chinese Spring'

M58484302

PCT/DE96/01185

These markers are distinguished by a high degree of polymorphism between different wheat varieties or lines and usually detect several alleles per genetic locus in different wheat lines.

They can therefore be used for DNA fingerprinting, species identification, relationship or similarity studies, characterization of cytological lines, such as deletion lines, substitution lines, addition lines, etc. and all forms of genetic ^{mapping}, including mapping of individual genes and quantitative ~~transferring~~ ^{transferring} features (QTLs). In addition, their use is also very suitable for automation and it is possible to carry out the detection of the products with nonradioactive methods.

With the help of ^{these} ~~this inventive~~ ^{marker} marker, the possibility is provided, for example, of differentiating almost all European wheat lines.

The invention is described in greater detail below by means of examples.

1. Amplification of the Microsatellite Markers

The microsatellite markers are amplified according to the following protocol:

10 mM tris-HCl, pH 8

50 mM KCl

1.5 mM MgCl₂ (in a few exceptional cases 3 mM MgCl₂)

0.01% (w/v) gelatin

0.2 mM of each desoxynucleotide

250 nM of each primer (in each case to the left and right of a pair)

1 - 2 units taq polymerase

50 - 150 ng matrixes (template) DNA

are amplified in a volume of 25 or 50 μ L according to the following profile:

92°C	3 minutes
92°C	1 minute (denaturing phase)
60°C	1 minutes (annealing phase)
72°C	2 minutes (elongation phase)
72°C	10 minutes (extension phase)

The amplification takes place in a Perkin Elmer 9600 with lid heating or in an MJ Research Thermocycler without lid heating. In this apparatus, a layer of mineral oil is placed over the reactions. The temperature of the annealing phase depends on the melting point (T_m) of the primer and in some cases even is 50°C or 55°C.

2. Separation of the Microsatellite Markers on Polyacrylamide Gels, Which Are Not Denaturing

The PCR reactions are mixed with 1/10 volume of stop buffer (0.02 M tris acetate of pH 8.1, 0.025 M sodium acetate, 0.02 M EDTA, 70% glycerin, 0.2% SDS, 0.6% bromphenol blue, 0.6% xylene cyanol) and in each case 25 μ L are separated in 10% polyacrylamide gels (1.5 mm thick, 18 cm long).

Formulation for polyacrylamide gel (10%):

25 mL stock acrylamide solution (19 g acrylamide, 1 g bisacrylamide, diluted to 100 mL with water)

10 mL 5X TBE (1X TBE = 0.09 M tris borate of pH 8.3, 0.002 M EDTA

15 mL water

are mixed and the polymerization is started by the addition of 220 µL of ammonium persulfate (10%, freshly prepared) and 20 µL of TEMED. Immediately after the addition, the mixture is poured into the sealed gel mold and the comb for forming pockets is inserted. The polymerization is completed after about 1 hour. The gel is placed in the gel chamber and a preliminary run is carried out without samples for about 30 minutes at 150 volts in 1X TBE. After that, the samples are loaded (25 µL of each) and the separation is carried out for 14 - 16 hours at 100 volts.

After the electrophoresis is completed, the gel is stained ~~for about~~ minutes in ethidium bromide (1 - 2 drops of 10 mg/mL in 1 liter of water) and the fragments are made visible by a UV transilluminator and documented.

3. Separation of Microsatellite Markers on Denaturing Gels

For the separation of the amplified fragments on denaturing gels, an automatic laser fluorescence (A.L.F.) sequencer (Pharmacia), for example, is used. In order to enable the fragments to be detected by means of a laser, one primer per pair is marked at the 5' end with fluorescein. Per PCR reaction, 0.3 to 1.5 microliters are mixed with 2.5 microliters of stop buffer (deionized formamide; 5 mg/mL dextran blue), denatured (1 minute; 90°C) and loaded onto the gel. Gel plates with a 9 cm separation distance are used, as recommended by the manufacturer for the fragment analysis. The gel solution contains 6.5% Long-Ranger (AT Biochem), 7M urea and 1.2X TBE buffer. The gels are 0.35 or 0.5 mm thick. The conditions for the gel run are 600 V, 40 mA, 50 W, 0.84 s data collection interval and 2 mW laser energy. The gel runs are ended after about 80 to 90 minutes. This is sufficient for detecting fragments up to a size of 300 bp. A gel can be used for four or five runs. For each gel

•158484302

16

PCT/DE96/01185

run, a data set is obtained. With this data set and by means of internal size standards, the exact fragment sizes are determined in the computer program Fragment Manager (Pharmacia) and thus the smallest size differences of a base pair are determined.

06 983 985 986 987 988
989 980 981 982

WMS-Number	WMS-Primer links	WMS-Primer left	WMS-Primer right	WMS-Primer rechus	Repeat Type Repcat-Typ
WMS052	5' CTA TGA GGC GGA GGT TGA AG 3'	5' TGC GGT GCT CTT CCA TTT 3'	5' CGA TCA AGT AGT TGA AAG CGC 3'	5' TCA TGG ATG CAT CAC ATC CT 3'	GTTimp
WMS055	5' GCA TCT GGT ACA CTA GCT GCC 3'	5' CGA TCA AGT AGT TGA AAG CGC 3'	5' GAA AAA AAT TGC ATA TGA GCC C 3'	5' CGA TCA AGT AGT TGA AAG CGC 3'	CTImp
WMS057	5' TCG ATT CTG AAA GGT TCA TCG 3'	5' GAA AAA AAT TGC ATA TGA GCC C 3'	5' GCA TTG ACA GAT GCA CAC G 3'	5' GAA AAA AAT TGC ATA TGA GCC C 3'	AAAAAimp
WMS058	5' TCT GAT CCC GTG AGT GTA ACA 3'	5' GCA TTG ACA GAT GCA CAC G 3'	5' CGC CCT GGG TGA ATA GT 3'	5' CAA CCC TCT TAA TTT TGT TGG G 3'	CA
WMS060	5' TGT CCT ACA CGG ACC ACG T 3'	5' CGC CCT GGG TGA ATA GT 3'	5' CCT CCT AGA TGG GAG AAG GG 3'	5' CAA CCC TCT TAA TTT TGT TGG G 3'	CA
WMS063	5' TCG ACC TGA TCG CCC CTA 3'	5' ACC ACA CAA ACA AGG TAA GCG 3'	5' CTC CCT AGA TGG GAG AAG GG 3'	5' GCC CAT TAC CGA GGA CAC 3'	GAA,CA,TA
WMS067	5' ACC ACA CAA ACA AGG TAA GCG 3'	5' AGG CCA GAA TCT GGG AAT G 3'	5' CTC CCT AGA TGG GAG AAG GG 3'	5' CAA GTG GAG CAT TAG GTA CAC G 3'	CA
WMS068	5' AGT GGC TGG GAG AGT GTC AT 3'	5' AGT GGC TGG GAG AGT GTC AT 3'	5' AGT GGC TTC TCT CTC AA 3'	5' AGT GGC TTC TCT CTC AA 3'	GA
WMS070	5' GGC AGA GCA GGC AGA CTC 3'	5' GGC AGA GCA GGC AGA CTC 3'	5' TCC ATX GGC AAA ACE CGC 3'	5' AAT GCA AAC TGA AAA ACE CGC 3'	GT
WMS071	5' ACA AAG GTA AGC AGC ACC TG 3'	5' ACC CTC TTG CCC GTG TTG 3'	5' GCC ATA TTT GAT GAC GCA TA 3'	5' GCC ATA TTT GAT GAC GCA TA 3'	GT
WMS077	5' ACG TTA GAA GGT GCA ATG GG 3'	5' AGT GGC TTC TCT CTC AA 3'	5' GCC ATA TTT GAT GAC GCA TA 3'	5' AGT TGG CTT GAT TAT TG 3'	CA,GA
WMS082	5' CAC TAC AAC TAT GCG CTC GC 3'	5' TCC ATX GGC AAA ACE CGC 3'	5' GCC ATA TTT GAT GAC GCA TA 3'	5' AGT TGG CTT GAT GAC ACA ATT GGG ATG G 3'	GT,GAmimp
WMS088	5' GAT CAA ACA CAC ACC CCT CC 3'	5' AAG ATG GAC GTA TGC ATC ACA 3'	5' AAT AAG GAC ACA ATT GGG ATG G 3'	5' GGT TCT AGG AGC AAG AAC AC 3'	GT
WMS095	5' TCT CCC ATC CAA CGC CTC 3'	5' CTG TTC TTG CGT GGC ATT AA 3'	5' ACC TGA TCA GAT CCC ACT CG 3'	5' TGC ACA CTT AAA TTA CAT CGG C 3'	CT
WMS099	5' ATT AAT ACC TGA GGG AGG TGC 3'	5' TCT GTC TCT CTC CGA CTG 3'	5' ACC TGA TCA GAT CCC ACT CG 3'	5' ACC TGA TCA GAT CCC ACT CG 3'	CT,GT
WMS102	5' CTA AAC ACG ACA GCG GTG G 3'	5' ATT CGA GGT TAG GAG GAA GAG G 3'	5' GAT ATG TGA GCA GCG GTC AG 3'	5' GAG GGT CGG CCT ATA AGA CC 3'	CTImp
WMS106	5' ACA AAC AGA AAA TCA AAA CCC G 3'	5' ATC CAT CGC CAT TGG AGT G 3'	5' GAT ATG TGA GCA GCG GTC AG 3'	5' GAT TAG TCA AAT GGA ACA CCC C 3'	GT
WMS107	5' GAT GTC GCC ACT TGA GCA TG 3'	5' ATC CAT CGC CAT TGG AGT G 3'	5' GAG GGT CGG CCT ATA AGA CC 3'	5' CAT GTC TCA ACC ACC CAC AG 3'	CA
WMS108	5' TGA CTA ACA TCC TTT GTC ACG C 3'	5' ATT CGA GGT TAG GAG GAA GAG G 3'	5' GAT TAT ACT GGT GCC GAA AC 3'	5' GAT TAT ACT GGT GCC GAA AC 3'	GTTimp
WMS111	5' GAT TAT ACT GGT GCC GAA AC 3'	5' TCC TCT ACA AAC AAA AAC AAC AC 3'	5' TCC TCT ACA AAC AAA AAC AAC AC 3'	5' TCC TCT ACA AAC AAA AAC AAC AC 3'	CT,CA
WMS112	5' GAT TAT ACT GGT GCC GAA AC 3'	5' TCC TCT ACA AAC AAA AAC AAC AC 3'	5' TCC TCT ACA AAC AAA AAC AAC AC 3'	5' TCC TCT ACA AAC AAA AAC AAC AC 3'	CA
WMS113	5' GAT TAT ACT GGT GCC GAA AC 3'	5' TCC TCT ACA AAC AAA AAC AAC AC 3'	5' TCC TCT ACA AAC AAA AAC AAC AC 3'	5' TCC TCT ACA AAC AAA AAC AAC AC 3'	CA
WMS114	5' GAT TAT ACT GGT GCC GAA AC 3'	5' TCC TCT ACA AAC AAA AAC AAC AC 3'	5' TCC TCT ACA AAC AAA AAC AAC AC 3'	5' TCC TCT ACA AAC AAA AAC AAC AC 3'	CA
WMS118	5' GAT TAT ACT GGT GCC GAA AC 3'	5' TCC TCT ACA AAC AAA AAC AAC AC 3'	5' TCC TCT ACA AAC AAA AAC AAC AC 3'	5' TCC TCT ACA AAC AAA AAC AAC AC 3'	CA
WMS119	5' GAT TAT ACT GGT GCC GAA AC 3'	5' TCC TCT ACA AAC AAA AAC AAC AC 3'	5' TCC TCT ACA AAC AAA AAC AAC AC 3'	5' TCC TCT ACA AAC AAA AAC AAC AC 3'	CA
WMS120	5' TCC TCT ACA AAC AAA AAC AAC AC 3'	5' TCC TCT ACA AAC AAA AAC AAC AC 3'	5' TCC TCT ACA AAC AAA AAC AAC AC 3'	5' TCC TCT ACA AAC AAA AAC AAC AC 3'	CA
WMS121	5' TCC TCT ACA AAC AAA AAC AAC AC 3'	5' TCC TCT ACA AAC AAA AAC AAC AC 3'	5' TCC TCT ACA AAC AAA AAC AAC AC 3'	5' TCC TCT ACA AAC AAA AAC AAC AC 3'	CA

M58484302

PCT/DE96/01185

WO 97/01567

19

MS122	5' GGG TGG GAG AAA GGA GAT G 3'	5' AAA CCA TCC TCC ATC CTG G 3'	CT, CA
MS124	5' GCC ATG GCT ATC ACC CAG 3'	5' ACT GTC CGG TGC AAT TTG AG 3'	CT, GTImp
MS126	5' CAC ACG CTC CAC CAT GAC 3'	5' GTC GAG TTG ATG CGG GAG G 3'	CA
MS128	5' AGC ACA TTT TAA CAC AGA TA 3'	5' ATC TGT GAA ATT TTG AAA AC 3'	CA
MS129	5' TCA GTG GGC AAG CTA CAC AG 3'	5' AAA ACT TAG TAG CCG CGT 3'	GTimep
MS130	5' AGC TCT GCT TCA CGA GGA AG 3'	5' CTC CTC TTT ATA TCG CGT CCC 3'	GT
MS131	5' ATC CCC CAC CGA TTC TTC TC 3'	5' AGT TCG TGG GTC TCT GAT GG 3'	CT
MS132	5' TAC CAA ATC GAA ACA CAT CAG G 3'	5' CAT ATC AAG GTC TCC TTC CCC 3'	GA, GAA
MS133	5' ATC TAA ACA AGA CGG CGG TG 3'	5' ATC TGT GAC AAC CGG TGA GA 3'	CT
MS134	5' CAT GGA ACT TAG ACA GAA TTG 3'	5' CAG TAC TTG GTA CTG AAC AGG 3'	CA
MS135	5' TGT GAA CAT CGT TTT GAA AAG G 3'	5' ACA CTG TCA ACC TGG CAA TG 3'	GA
MS136	5' GAC AGC ACC TTG CCC TTT G 3'	5' CAT CGG CAA CAT GCT CAT C 3'	CT
MS140	5' ATG GAG ATA TTT GGC CTA CAA C 3'	5' CTT GACT TGC AAG GCG TGA CA 3'	CT
MS144	5' TTT GCT GTG GTA CGA AAC ATA C 3'	5' ACT CAC AAA TGT CTA ATA AAA C 3'	GT
MS146	5' CCA AAA AAA CTG CCT GCA TG 3'	5' CTC TGG CAT TGC TCC TTG G 3'	GAimp
MS148	5' GTG AGG CAG CAA GAG AGA AA 3'	5' CAA AGCTTG ACT CAG ACC AAA 3'	CA
MS149	5' CAT TGT TTT CTG CCT CTA GCC 3'	5' CTA GCA TCG AAC CTG AAC AAG 3'	GA
MS153	5' GAT CTC GTC ACC CGG AAT TC 3'	5' TGG TAG AGA AGG ACG GAG AG 3'	GA
MS154	5' TCA CAG AGA AGG GAG GG 3'	5' ATG TGT ACA TGT TGC CTG CA 3'	GA
MS155	5' CAA TCA TTT CCC CCT CCC 3'	5' AAT CAT TGG AAA TCC ATA TGC C 3'	CT
MS156	5' CCA ACC GTG CTA TTA GTC ATT C 3'	5' GAG TGA ACA CAC GAG GCT TG 3'	GT
MS157	5' GTC GTC GCG GTA AGC TTG 3'	5' CAA TGC AGG CCC TCC TAA C 3'	CT
MS159	5' GGG CCA ACA CTG GAA CAC 3'	5' GCA GAA GCT TGT TGG TAG GC 3'	GT
MS160	5' TTC AAT TCA GTC TTT GCT TGG 3'	5' CTG CAG GAA AAA AAG TAC ACC C 3'	GA
MS161	5' GAT CGA GTG ATG GCA GAT GG 3'	5' TGT GAA TTG CTT GGA CGT GG 3'	CT
MS162	5' AGT GGA TCG ACA AGG CTC TG 3'	5' AGA AGC AGC AAA GCC TTC CC 3'	CA
MS163	5' ACC TCG ACA GAC CTG GTA CG 3'	5' GTC TTT GTC ACC CGA TGG AC 3'	CT
MS164	5' ACA TTT CTC CCC CAT CGT C 3'	5' TGT TAA ACA ACA ATG CGG ATG CG 3'	CT

158484302

WO 97/01567

PCT/DE96/01185

20

WMS165	5' TGC AGT GGT CAG ATG TTT CC 3'	GA
WMS169	5' ACC ACT GCA GAG AAC ACA TAC G 3'	GA
WMS174	5' GGG TTC CTA TCT GGT AAA TCC C 3'	CT
WMS179	5' AAG TTG AGT TGA TGC GGG AG 3'	GT
WMS180	5' ATC CGC CTA AGG AAT AGT GT 3'	CT
WMS181	5' TCA TTG GTA ATG AGG AGA GA 3'	GA
WMS182	5' TGA TGT AGT GAG CCC ATA GGC 3'	CT
WMS186	5' GCA GAG CCT GGT TCA AAA AG 3'	GA
WMS189	5' AGG AGC AGG GGA ACG AAC 3'	CA
WMS190	5' GTG CTT GCT GAG CTA TGA GTC 3'	CT, GT
WMS191	5' AGA CTG TTG TTT GCG GGC 3'	CT
WMS192	5' GGT TTT CTC TCA GAT TGC GC 3'	CT, CA
WMS193	5' CTT TGT GCA CCT CTC TCT TCC 3'	CT
WMS194	5' GAT CTG CTC TAC TCT CCT CC 3'	CT
WMS195	5' AGG TGC CGT CGC GTG TAC 3'	CT
WMS197	5' GAG AAA GAG GTC TGG AGG TCG 3'	CT
WMS198	5' TTG AAC CGG AAG GAG TAC AG 3'	CA
WMS200	5' TCA ACG GAA CAG ATG AGC G 3'	CT
WMS203	5' CCC AAA GCA GCG CAA GC 3'	CA, GA
WMS205	5' CGA CCC GGT TCA CTT CAG 3'	CT
WMS210	5' TGC ATC AAG AAT AGT GTG GAA G 3'	GA
WMS212	5' AAG CAA CAT TTG CTG CAA TG 3'	CT
WMS213	5' TGC CTG GCT CGT TCT ATC TC 3'	GA
WMS218	5' CGG CAA ACG GAT ATC GAC 3'	CT
WMS219	5' GAT GAG CGA CAC CTA GCC TC 3'	GAimp
WMS224	5' TGA GTC CAG CAC TGC TGC 3'	CT
WMS228	5' TCA TAT GCA CCT CTT TCC TAG G 3'	CT, CA
WMS31	5' AGC TCG GGA TGA AGC GTG 3'	GAimp

WO 97/01567

58484302

22

MS275	5' AAT TTT CTT CCT CAC TTA TTCTT 3'	5' AAC AAA AAA TTA GGG CC 3'	CT
MS276	5' ATT TGC CTG AAG AAA ATA TT 3'	5' AAT TTC ACT GCA TAC ACA AG 3'	CT
MS278	5' GTT GCT TCA TGA ACG CTC AA 3'	5' CTG CCC AA TTT CTC CAC TC 3'	GTTimpGAimp
MS281	5' CGG CCA TAT TTCT TGT AAG TAT GC 3'	5' GCA GGT AAT GGC CGG AC 3'	GT
MS282	5' TTG GCC GTG TAA GGC AG 3'	5' TCT CAT TCA CAC ACA CTA GC 3'	GA
MS284	5' AAT GAA AAA ACA CTT GCG TGG 3'	5' GCA CAT TTT TCA CTT TCG GG 3'	GA
MS285	5' ATG ACC CTT CTG CCA AAC AC 3'	5' ATC GAC CGG GAT CTA GCC 3'	GA
MS291	5' CAT CCC TAC GCC ACT CTGC C 3'	5' AAT GGT ATC TAT TCC GAC CCG 3'	CA
MS292	5' TCA CCG TGG TCA CCG AC 3'	5' CCA CCG AGC CGA TAA TGT AC 3'	CT
MS293	5' TAC TGG TTC ACA TTG GTG CG 3'	5' TCG CCA TCA CTC GTT CAA G 3'	CA
MS294	5'-GGA-TTG-GAG-TTA AGA GAG AAC CG 3'	5' GCA GAG TGA TCA ATG CCA GA 3'	GAimp
MS295	5' GTG AAG CAG ACC CAG-AAC AC 3'	5' GAC GGC TGC GAC GTA GAG 3'	GA
MS296	5' AAT TCA ACC TAC CAA TCT CTG 3'	5' GCC TAA TAA ACT GAA AAC GAG 3'	CT
MS297	5' ATC GTC ACG TAT TTT GCA ATG 3'	5' TGC GTA AGT CTA GCA TTT TCT G 3'	GT, GA
MS299	5' ACT ACT TAG GCC TCC CGC C 3'	5' TGA CCC ACT TGC AAT TCA TJC 3'	GA, TAG
MS301	5' GAG GAG TAA GAC ACA TGC CC 3'	5' GTG GCT GGA GAT TCA GGT TCS 3'	GA
MS302	5' GCA AGA AGC AAC AGC AGT AAC 3'	5' CAG ATG CTC TTC TCT GCT GG 3'	CT
MS304	5' AGG AAA CAG AAA TAT CGC GG 3'	5' AGG ACT GTG GGG AAT GAA TG 3'	GA
MS311	5' TCA CGT GGA AGA CGC TCC 3'	5' CTA CGT GCA CCA CCA TTT TG 3'	GA
MS312	5' ATC GCA TGA TGC ACG TAG AG 3'	5' TTT GAC AAG TAC ACG AGT CTGC C 3'	CT, GT
MS313	5' CGG CCC TCA TTA AGT TTC AC 3'	5' TTC GGG ACT CTC TTC CCT G 3'	CT
MS314	5' AGG AGC TCC TCT GTG CCA C 3'	5' TGC GTG TGG TCC ACC TC 3'	AT, GT
MS316	5' CAT GGA CAT TTT ACC ACA AGA C 3'	5' CGG GTG CTG TGT GTA ATG AC 3'	CT
MS319	5' GGT TGC TGT ACA AGT GTT CAC G 3'	5' ATC TTT GCA AGG ATT GCC C 3'	GT, GA
MS320	5' CGA GAT ACT ATG GAA GGT GTG C 3'	5' TGT TGC ATG CGA TCA TGC 3'	GT, GAimp
MS321	5' CAA TGT GGA GAC GGT GTG C 3'	5' TGC AGA AAA CCA ACA AGG G 3'	GA
MS322	5' TCA CAA AAT GAT TTC TCA TCC G 3'	5' TTT TTA CGC GTC AAC GAC G 3'	CT
MS325	5' TTT CTT CTG TCG TTC TCT TCC C 3'	5' TTT TTA CGC GTC AAC GAC G 3'	CT

58 484302

MS328	5' GCA ATC CAC GAG AAG AGA GG 3'	5' CAC AAA CTC TTG ACA TGT GCG 3'	GT
MS330	5' TTG CTA TCC ATG TGC CAG AG 3'	5' ACA TGT TTC ATG CAG GTA GCC 3'	GTT
MS332	5' AGC CAG CAA GTC ACC AAA AC 3'	5' AGT GCT GGA AAG AGT AGT GAA GC 3'	GA
MS333	5' GCC CGG TCA TGT AAA ACG 3'	5' TTT CAG TTT GCG TTG AGC TTT G 3'	GA
MS334	5' AAT TTC AAA AAG GAG AGA GA 3'	5' AAC ATG TGT TTT TAG CTA TC 3'	GA
MS335	5' CGT ACT CCA CTC CAC ACG G 3'	5' CGG TCC AAG TGC TAC CTT CAG G 3'	GA, GCGT
MS336	5' CCC TTT AAT CTC GCT CCC TC 3'	5' GTC TCT TTC TCG TAC TTC CAG G 3'	CT
MS337	5' CCT CTT CCT CCC TCA CTT AGC 3'	5' TGCT TAA CTG GCC TTT GCC 3'	CT, CACT, CA
MS339	5' AAT TTT CTT CCT CAC TTA TT 3'	5' AAA CGA ACA ACC ACT CAA TC 3'	CT
MS340	5' GCA ATC TTC TTT CTG ACC ACG 3'	5' ACG AGG CAA GAA CAC ACA TG 3'	GA
MS341	5' TTC AGT GGT AGC GGT CGA G 3'	5' CCG ACA TCT CAT GGA TCC AC 3'	CT
MS342	5' TAT CCA GAG CAG ACG GAC G 3'	5' GGT CTA GCT TCG ACG ACA CC 3'	GT
MS344	5' CAA GGA AAT AGG CGG TAA CT 3'	5' ATT TGA GTC TGA AGT TTG CA 3'	GT
MS346	5' CAA GCA AGG TTT CGT TTT ATC C 3'	5' GCA TGG ATA GGA TGG EC-EC-3'	AT, GT
MS349	5' GGCTTTC CAG AAA ACA ACA GG 3'	5' ATC GGT GCG-TAC CAT CCT AC 3'	GA
MS350	5' ACC TCA TCC ACA TGT TCT ACG 3'	5' GCA TGG CTG CAG ATG CTA CGA AC 3'	GT
MS353	5' CCA TGT TGA GTA GGT TCA GCC 3'	5' CTT GGC CAG XAG CTA CGA AC 3'	GGGT, GT
MS356	5' AGC GTT CTT GGG AAT TAG AGA 3'	5' CCA ATC AGC CTG CAA CAA C 3'	GA
MS357	5' TAT GGT CAA AGT TGG ACC TCG 3'	5' AGG CTG CAG CTC TTC AG 3'	GA
MS358	5' AAA CAG CGG ATT TCA TCG AG 3'	5' TCC GCT GTT GTT CTG ATC TC 3'	GAimp
JMS359	5' CTA ATT GCA ACA GGT CAT GGG 3'	5' TAC TTG TGT TCT GGG ACA ATGG 3'	CT, CTRimp
WMS361	5' GTA ACT TGT TGC CAA AGG GG 3'	5' ACA AAG TGG CAA AAG GAG ACA 3'	GAimp
WMS368	5' CCA TTT CAC CTA ATG CCT GC 3'	5' AAT AAA ACC ATG AGC TCA CTT GC 3'	AT
WMS369	5' CTG CAG GCC ATG ATG ATG 3'	5' ACC GTG GGT GTT GTG AGC 3'	CTimp
WMS371	5' GAC CAA GAT ATT CAA ACT GGC C 3'	5' AGC TCA GCT TGC TTG GTA CC 3'	CA, GA
WMS372	5' AAT AGA GCC CTG GGA CTG GG 3'	5' GAA GGA CGA CAT TCC ACC TG 3'	GA
WMS374	5' ATA GTG TGT TGC ATG CTG TGT G 3'	5' TCT AAT TAG CGT TGG CTG CC 3'	GT
WMS375	5' ATT GGC GAC TCT AGC ATA TAC G 3'	5' GGG ATG TCT GTT CCA TCT TAG C 3'	CA

158484302

PCT/DE96/01185

WO 97/01567

24

WMS376	5' GGG CTA GAA AAC AGG AAG GC 3'	5' TCT CCC GGA GGG TAG GAG G 3'	CA, GA imp
WMS382	5' GTC AGA TAA CGC CGT CCA AT 3'	5' CTA CGT GCA CCA CCA TTT TG 3'	GA
WMS383	5' ACG CCA GAT CCG TAA AC 3'	5' GAC ATC AAT AAC CGT GGA TGG 3'	GT
WMS384	5' TTT TCA TTG TGC CCT CTA CT 3'	5' GCC AAG TTT CTT AGC TAG TTA A 3'	GT imp
WMS388	5' CTA CAA TTC GAA GGA GAG GGG 3'	5' CAC CGC GTC AAC TACT TTA AGC 3'	CT, CA, CA
WMS389	5' ATC ATG TCG ATC TCC TTG AGC 3'	5' TGC CAT GCA CAT TAG CAG AT 3'	CT, GT
WMS390	5' AAG TTT CAC ACA AGA TCT CTC C 3'	5' TGA CAA GTA CAC GAG TCT GC 3'	CA, GA
WMS391	5' ATA GCG AAG TCT CCC TAC TCC A 3'	5' ATG TGC ATG TCG GAC GC 3'	CA
WMS393	5' TCA TCT GCT ATT TGT GCT ACA 3'	5' TCA AAT ACA CCA ATG TGC C 3'	CA
WMS395	5' TAC AAC CGC AAG TAA TGC CA 3'	5' TAC CAA CAC CCT AGC CCT TG 3'	CA
WMS397	5' TGT CAT GGA TTA TTT GGT CGG 3'	5' CTG CAC TCT CGG TAT ACC AGC 3'	CT
WMS400	5' GTG CTG CCA CCA CTT GC 3'	5' TGT AGG CAC TGC TTG GGA G 3'	CA
WMS403	5' CGA CAT TGG CTT CGG TG 3'	5' ATA AAA CAG TGC GGT CCA GG 3'	CA
WMS408	5' TCG ATT TAT TTG GGC CACT G 3'	5' GTA TAA TTC GTT CAC AGC ACG C 3'	CA
WMS410	5' GCT TGA GAC CGG CAC AGT 3'	5' CGA GAC CCT GAG GGT CTA GA 3'	CA
WMS411	5' CCC ATA CGA TGA TGT GTT TCC 3'	5' CAA ACG GAA CAT GGT CCC 3'	CT
WMS412	5' ATC AAC AAG GTT TGT GTG TTG G 3'	5' ATG AAA CGC GAC CCT CCC 3'	GA
WMS413	5' TGC TTG TCT AGA TTG CTT GGG 3'	5' GAT CGT CTC GTC CTT GGC A 3'	GA
WMS415	5' GAT CTC CCA TGT CCG CC 3'	5' CGA CAG TCG TCA CTT GCC TA 3'	GA imp
WMS425	5' GAG CCC ACA AGC TGG CA 3'	5' TCG TTC TCC CAA GGC TTG 3'	CT
WMS427	5' AAA CTT AGA ACT GTA ATT TCA GA 3'	5' AGT GTG TTC ATT TGA CAG TT 3'	CA
WMS428	5' CGA GGC AGC GAG GAT TT 3'	5' TTC TCC ACT AGC CCC GC 3'	GA
WMS429	5' TTG TAC ATT AAG TTC CCA TTA 3'	5' TTT AAG GAC CTA CAT GAC AC 3'	CT
WMS434	5' ATG AGT TCC GCC AAA GAA TG 3'	5' AGG AAA TAC ACA AGT GGG ACA 3'	GT
WMS437	5' GAT CAA GAC TTT TGT ATC TCT C 3'	5' GAT GTC CAA CAG TTA GCT TA 3'	CT
WMS440	5' CCT ATG GTC TCC ATC ATG AGG 3'	5' TCA TGT CAA CTC AAG AAC ACG 3'	CT
WMS443	5' GGG TCT TCA TCC GGA ACT CT 3'	5' CCA TGA TTT ATA AAT TCC ACC 3'	CA, GA
WMS445	5' TTT GTT GGG GGT TAG GATTAG 3'	5' CCT TAA CAC TTG CTG GTA GTG A 3'	CT

M58484302

PCT/DE96/01185

WO 97/01567

25

MS448	5' AAA CCA TAT TGG GAG GAA AGG 3'	5' CAC ATG GCA TCA CAT TGG TG 3'	GA
MS455	5' ATT CGG TTC GCT AGC TAC CA 3'	5' ACG GAG AGC AAC CTG CC 3'	GTrimp
MS456	5' TCT GAA CAT TAC ACA ACC CTG A 3'	5' TGC TCT CTC TGA ACC TGA AGC 3'	GA
MS458	5' AAT GGC AAT TGG AAG ACA TAG C 3'	5' TTC GCA ATG TIG ATT TGG C 3'	CA
MS459	5' ATG GAG TGG TCA CAC TTT GAA 3'	5' AGC TTC TCT GAC CAA CTT CTC CTC G 3'	GA
MS469	5' CAA CTC AGT GCT CAC ACA ACG 3'	5' CGA TAA CCA CTC ATC CAC ACC 3'	CT
MS471	5' CGG CCC TAT CAT GGCT TG 3'	5' GCT TGC AAG TTC CAT TTT GC 3'	CA
MS473	5' TCA TAC GGG TAT GGT TGG AC 3'	5' CAC CCC CTT GTT GGT CAC 3'	GTrimp
MS476	5' ATG GGT TCG TAC TAA CAT CAG C 3'	5' TTG CTG GTA GCT TCA ATC CC 3'	CT, CA
MS480	5' TGC TGC TAC TTG TAC AGA GGA C 3'	5' CCG AAT TGT CCG CCA TAG 3'	CT
MS484	5' ACA TCG CTC RTC ACA AAC CC 3'	5' AGT TCC GGT CAT GGC TAG G 3'	CA
MS494	5' ATT GAA CAG GAA GAC ATC AGG G 3'	5' TTC CTG GAG CTG TCT GGC 3'	GA
MS495	5' GAG AGC CTC GCG AAA TAT AGG 3'	5' TGC TTC TGG TGT TCC TTC G 3'	GTrimp
MS497	5' GTA GTG AAG TCA AGG GCA TT 3'	5' CCG AAA GTT GGG TGA TAT AC 3'	GA
MS499	5' ACT TGT ATG CTC CAT TGA TTG-G 3'	5' GGG GAG TGG AAA CTG CAT AA 3'	CA
VMSS501	5' GGC TAT CTC TGG CGC TAA AA 3'	5'-TCC ACA AAC AAG TAG CGC C 3'	GT
VMSS512	5' AGC CAC CAT CAG CAA AAA TT 3'	5' GGT CTG TTC ATG CCA CAT TG 3'	CA
VMSS513	5' ATC CGT AGC ACC TAC TGG TCA 3'	5' CCT TCC TAG TAA GTG TGC CTC A 3'	GTrimp
VMSS515	5' AAC ACA ATG GCA AAT GCA GA 3'	5' CAG GGT GGT GCA TGC AT 3'	CA
VMSS518	5' AAT CAC AAC AAG GCG TGA CA 3'	5' TCA ACT TCT TGG CCT CCA TC 3'	CT
WMSS530	5' AAA TAG GAC AAC CCA CGG C 3'	5' TCA CTC GCA CTC GAT AGG C 3'	GT
WMSS532	5' ACT GCG TGT GCC TAC AAT TG 3'	5' GTT GCT TTA GGG GAA AAG CC 3'	CT, CA
WMSS533	5' AAG GCG AAT CAA ACG GAA TA 3'	5' GCC ACT TTT GTG TCG TTC CT 3'	CA, TA
WMSS537	5' ACA TAA TGC TTC CTG TGC ACC 3'	5' GTT GCA TGT ATA CGT TAA GCG G 3'	GTrimp
WMSS538	5' GCA TTT CGG GTG AAC CC 3'	5' AGG CAT GGA TAG AGG GGC 3'	CT, ATCT, CT
WMS540	5' TCT CGC TGT GAA ATC CTA TTTC 3'	5' AGG ATT CCA ATC CTT CAA ATT T 3'	CT, GT
WMS544	5' TAG AAT TCT TTA TGG GGT CTG C 3'	5' CAT TGT GTG TGC AAG GCA C 3'	
WMS550	5' CCC ACA AGA ACC TTG GAA GA 3'		

58484302

PCT/DE96/01185

WO 97/01567

26

MS554	5' TGC CCA CAA CGG AAC TTG 3'	5' GCA ACC ACC AAG CAC AAA GT 3'	CT, GTimp
MS565	5' GCG TCA GAT ATG CCT ACC TAG G 3'	5' AGT GAG TTA GCC CTG AGC CA 3'	CA
MS566	5' TCT GTC TAC CCA TGG GAT TTG 3'	5' CTG GCT TCG AGG TAA GCA AC 3'	CA, TA
MS569	5' GGA AAC CTTA TTG ATT GAA AT 3'	5' TCA ATT TTG ACA GAA GAA TT 3'	GT
MS570	5' TCG CCT TTT ACA GTC GGC 3'	5' ATG GGT AGC TGA GAG CCA AA 3'	CT, GT
MS573	5' AAG AGA TAA CAT GCA AGA AA 3'	5' TTC AAA TAT GTG GGA ACT AC 3'	CA
MS577	5' ATG GCA TAA TTT GGT GAA ATT G 3'	5' TGT TTC AAG CCC AAC TTCTAT T 3'	CA, TA
MS582	5' AAG CAC TAC GAA AAT ATG AC 3'	5' TCT TAA GGG GTG TTA TCA TA 3'	CA
MS583	5' TTC ACA CCC AAC CAA TAG CA 3'	5' TCT AGG CAG ACA CAT GCC TG 3'	CA
MS588	5' GAT CCC CAA TTG CAT GTT G 3'	5' CTT GCA ACT OGG GGA CAC 3'	GT